Use of attenuated viruses

Attenuated viruses are used as **vaccines**. It is the oldest and most effective method of vaccination. Their discovery is also linked to the search for effective ways of prevention against previously widespread and fatal diseases, some of which have now been completely eradicated worldwide, eradicated in developed countries, or at least reduced in incidence and mortality.

The use of live, albeit attenuated, viruses carries certain **risks**. The greatest of these is the possibility of reversion, i.e. a return to virulence and the emergence of disease. There are also more storage requirements for vaccines, as cold chain disruption can lead to loss of antigenic properties. Several scientific studies are now underway to try to design a way of producing safer vaccines by controlling replication and virulence, which could, for example, help in the eradication of polio virus.

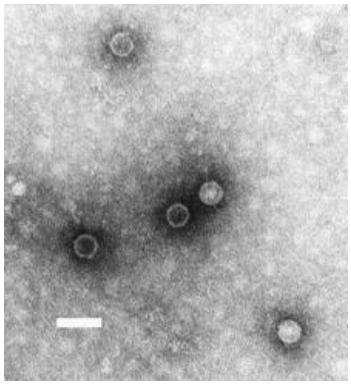
History of use

The first vaccination with attenuated vaccinia virus, the causative agent of **cowpox**, was carried out in 1796 by Edward Jenner as a defence against variola virus, the causative agent of smallpox. Thanks to an aggressive vaccination campaign, smallpox was eradicated in 1980. Today, smallpox is no longer routinely vaccinated against. However, smallpox virus remains available in some laboratories, raising concerns about its possible use for bioterrorism.

Another success in the war against viral diseases was the use of **Sabin's polio vaccine**. The vaccine contains 3 attenuated strains called 1, 2, 3 and is administered orally (OPV, oral polio vaccine). In addition to this live attenuated vaccine, there is a **Salk's vaccine** containing killed viruses which is administered by injection (IPV); however, it does not induce mucosal intestinal immunity. Although a child vaccinated with IPV is protected from disease, the child can pass on polio virus if he/she comes into contact with it.

OPV has the advantage of inducing a stronger immune response after the first dose. The vaccinated person also excretes the weakened viruses in the stool, which can trigger an immune response in unvaccinated people who come into contact with it. The use of OPV, however, carries the risk of mutation and reversion of the attenuated virus, and thus the development of disease in vaccinated persons. According to the WHO, around 60 children a year fall ill as a result of vaccination[1], which can spread the mutated virus further to the unvaccinated. The largest outbreak of poliomyelitis caused by a vaccine strain occurred in 2005 in Nigeria and the virus is still circulating. Despite these isolated cases, poliomyelitis has been successfully eradicated in developed countries and only three endemic areas remain - Afghanistan, Nigeria and Pakistan. Worldwide, 650 cases were recorded in 2011 and 223 cases in 2012.[2]

The **WHO**-sponsored global campaign to eradicate poliomyelitis virus began in 1988 and was originally planned to end by 2000. However, the goal has still not been achieved. Wild type 2 virus has been eradicated, but vaccine-derived poliovirus (VDPV) infections remain a problem. In 90% of cases, it is a reversion-derived type 2 virus. A possible solution is to remove type 2 from the oral vaccine and introduce the current Salk vaccine, which protects against all three types, but this is a costly procedure. In developed countries, there has been a move to inactivated virus vaccination (IPV), which has been used in Czech Republic since 2007 and in the USA since 2000.



Poliovirus (negatively stained cell culture preparation; transmission electron microscopy; scale bar 50 nm)

Prospects for the future

The traditional method of attenuating viruses by repeated passaging on cell cultures under suboptimal conditions carries certain risks, and scientific teams are trying to find ways to obtain a non-pathogenic strain with preserved immunogenicity in a rational way. With a deeper understanding of the molecular nature of viruses, in particular the discovery of pathogenicity- and immunogenicity-associated genes, possible routes to safer vaccines are emerging. However, research is still being carried out in animal models but the possibility of its use in human medicine is still far beyond the horizon.

Four new methods appear most promising:

- increasing replication accuracy,
- codon deoptimization,
- the use of miRNAs,
- the use of nucleases with zinc finger motifs.

The high mutation rate of RNA viruses is largely due to the high error rate of viral RNA polymerase, which is approximately **0.1-10 mutations/genome replication** of about 10,000 nucleotides, which is several orders of magnitude higher than that of DNA viruses. This fact allows for very rapid evolution and adaptation, and as a result, a reduction in polymerase error rate will reduce population diversity and lead to attenuation in the animal model.

A team from the University of California, San Francisco, led by M. Vignuzzi[3], performed the assays on poliomyelitis virus polymerase using *ribavirin*, an analogue of nucleotide bases. Polymerase variants with mutations in the 64th amino acid in the chain, which plays a key role in controlling the amino acids inserted during transcription, were found to be resistant even to high concentrations of this analogue. Variants with 6 possible amino acids at position 64 - glycine, valine, alanine, serine, threonine and leucine - show low error rates, correlating with ribavirin resistance.

When tested, variants with a change at position 64 showed a reduced mutation rate during transcription (wild type - 5.32/genome, position 64 - AK 2.15-2.96) and even after prolonged passaging, viral strains with the mutation showed **reduced population diversity**. Thus, mutations at position 64 are stable. The reduced population diversity of the virus greatly reduces pathogenicity and the reduced missense variants are strongly attenuated and will not allow infection to develop in the central nervous system of the mouse but will not affect replication in other tissues and thus allow the development of an immune response[4].

The approach described above could be applied to other *picornaviruses*, but for other RNA viruses, it is necessary to first identify the key amino acid residues whose replacement would lead to reduced polymerase error rates. In September 2011, a study published by scientists at the Institut Pasteur in Paris, led by Lark L. Coffey, described the successful attenuation of chikungunya virus arbovirus (CHIKV) by replacing one AK in the polymerase chain, leading to a reduction in error rate comparable in percentage to the study on poliovirus. The experiment was performed in **a natural model of infection** - transmission from mosquito to a vertebrate mouse, which naturally expresses receptors for this virus. The authors further hypothesised that similar results could be achieved with other arboviruses and could lead to the development of genetically stable vaccines, thus preventing millions of human infections per year with CHIKV.

Other new methods allow for the production of attenuated strains by inserting **synonymous codons** into the genome, but these are less preferred by the host cell, thus increasing translation time and producing fewer viral progeny - the virus is strongly attenuated without affecting immunogenicity and is genetically stable.

By inserting a sequence binding a specific miRNA into the virus genome, translation of a specific gene or the entire genome can be stopped by binding a complementary miRNA expressed by the host cell. This principle is the **normal regulatory pathway** by which the cell regulates gene production, but it runs into problems in its use for attenuation in that the insertion miRNA sequence is transcribed only in certain tissues and can accumulate mutations.

Another method uses *zinc fingers*, specific DNA-binding domains that can serve as negative transcription factors or, by fusing with nuclease, can cleave a specific stretch of viral DNA. Their use is limited to non-integrating DNA viruses, as excision of integrated viral DNA could lead to chromosomal breaks in the host DNA.

Use of attenuated vaccines in the Czech Republic

In our country, according to the current legislation, vaccination using live attenuated viruses is **compulsory against measles, rubella and mumps**, formerly also against **polio**. Vaccines against chickenpox and diarrhoeal diseases caused by rotavirus are recommended but not compulsory.

A vaccine against Herpes zoster virus, recommended for individuals over 50 years of age, has been available since 2006 and reduces the incidence of shingles by 50% compared to a placebo group.

The yellow fever vaccine is mandatory for travel to endemic areas, countries requiring an international vaccination card and for persons handling infectious material.

Sources

Related articles

- Acute anterior poliomyelitis
- Regular vaccination in the Czech Republic
- Breakdown of vaccinations in the Czech Republic
- Active immunisation

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